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Evaluation of antibody response to coronavac 600 u/0.5 Ml (sinovac life science, beijing, china) vaccine administration in elderly individuals

Antibody response to coronavac in elderly individuals

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Abstract
Aim: In this study, we aimed to evaluate the presence of anti-SARS-CoV-2 antibodies before vaccination and the antibody response after CoronaVac vaccination administered in two doses 28 days apart in elderly individuals over 60 years of age who were planned to be vaccinated against COVID-19.
Material and Methods: Antibody levels were evaluated by taking blood samples from participants aged 60 years and over who were vaccinated with inactivated COVID-19 whole virus vaccine in the vaccination outpatient clinic before the first dose of vaccine, 28 days after vaccination (before the second dose), 2 months and 4 months.
Results: Of the 81 participants, 51.9% (n=42) were female, 48.1% were male, the mean age was 67.2±4.6 years, and 48.1% (n=39) had at least one comorbidity. It was found that both seropositivity rates and antibody titres of the participants increased in the first and second months, but in the fourth month, although there was no increase in antibody titres, the mean antibody level decreased compared to the second month. Antibody seropositivity increased both in the 1st month, 2nd month and 4th month compared to the pre-vaccination period. Antibody seropositivity at 2 and 4 months was 100.0% in individuals who had all antibody measurements completed at both 2 (n=49) and 4 (n=12) months.
Discussion: Vaccination against SARS-CoV-2 with CoronaVac in individuals over 60 years of age induces an effective antibody response, but additional doses are required for the sustainability of the antibody response.

Keywords
COVID-19, Antibody, Age 60, CoronaVac, Vaccine

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Introduction

The outbreak of "Coronavirus Disease 19 (COVID-19)" caused by "Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)", which has led to millions of deaths and devastating socio-economic consequences worldwide, is still continuing its effect as a pandemic [1]. SARS-CoV-2 is a zoonotic, positive polarity RNA virus and has four important structural proteins that play a role in viral infection and replication: spike (S), envelope (E), membrane (M) and nucleocapsid (N). Coronaviruses are enveloped positive-stranded RNA viruses that enter a host cell through a fusion of the envelope lipid bilayer with the target cell membrane. This first critical step of viral infection is catalysed by the trimeric spike (S) protein, which decorates the virion surface as the main antigen and induces neutralizsing antibody responses. Therefore, the protein is an important target for the diagnosis, treatment and development of vaccines [2,3].

The CoronaVac vaccine produced by Sinovac Life Science in China contains inactivated SARS- CoV-2 virus and is adjuvanted with aluminium hydroxide [4]. Phase III trials were conducted in various parts of the world and efficacy results ranged between 50.7% (Brazil), 65.30% (Indonesia) and 83.5% (Turkey) [5]. The vaccine induces both innate and adaptive immune responses through various mechanisms, simultaneously generating a cellular response (T cells) and an antibody response (B cells) resulting in the production of antibodies directed against various SARS-CoV-2 antigens. After vaccination, the body is expected to produce specific antibodies against the SARS-CoV-2 S protein, which can neutralizse the virus and prevent it from binding to its specific receptor (Angiotensin Converting Enzyme-2, ACE 2 receptor) [6].

The SARS-CoV-2 S protein is a transmembrane fusion glycoprotein consisting of S1 and S2 subunits with a size of 180-200 kDa and a total length between 1273 and 1300 amino acids. It is the basic unit of the virus that recognizses and binds to the host cell receptor ACE-2 [7]. The S protein, the only protein responsible for entry into the host cell, is the main antigen that elicits neutralizsing antibodies. Therefore, the SARS-CoV-2 S protein appears to be the best target site in antibody detection tests [8]. Quantitative tests detecting anti-SARS-CoV-2 antibodies can help monitor individual antibody titre and the long-term course of antibody response by measuring the specific antibody response to vaccines [9].

In SARS-CoV-2 infections, neutralizsing antibodies that prevent virus entry, fusion and exit from the cell play an important role in the antibody-mediated killing of the virus. Since the antibody response against S-protein is associated with neutralizsing antibodies, the main aim is to obtain an anti-S antibody response in the immune response that develops after vaccination [10]. Epidemiological studies have reported that neutralizsing antibody titres vary greatly in convalescent serum samples and may be related to various factors (e.g. age, sex, disease severity and the number of days after infection). Therefore, it is unclear whether vaccine-induced antibody levels will persist and whether long-term memory will affect the sensitivity and pathogenesis of T cells to SARS-CoV-2 infection [11]. Studies have reported a statistically significant decrease in vaccine efficacy against symptomatic COVID-19 in the elderly in parallel with age after vaccination [12-15]. More evidence is required to determine the efficacy of the vaccine, especially in elderly individuals with a high risk of mortality due to this disease.

In this study, we aimed to evaluate the presence of anti- SARS-CoV-2 antibody before vaccination and the antibody response after the CoronaVac vaccination administered 28 days apart in elderly individuals over 60 years of age who were planned to be vaccinated against COVID-19.

Material and Methods

Participants

Participants aged 60 years and older who were vaccinated with inactivated COVID-19 whole virus vaccine in Afyonkarahisar Health Sciences University vaccine outpatient clinic were included in the study. None of the participants had a history of COVID-19 during the pandemic period and none of them had SARS-CoV-2 positivity by reverse transcriptase polymerase chain reaction (RT-PCR) method in nasopharyngeal swab samples in the pre-vaccination period. The vVaccine administration was performed intramuscularly in the left arm with two doses of CoronaVac 600 U/0.5 mL (Sinovac Life Science, Beijing, China). Blood samples were collected from the participants four times before the first dose of vaccine, on the 28th day after vaccination (before the second dose), in the 2nd month and 4th months, and antibody levels were evaluated (flow chart is given in Figure 1).

Laboratory Analyses

Venous blood samples were collected from the patients in a 10 ml Vacutainer SST tube with gel (Becton Dickinson, France). After waiting for 30 min, it was centrifuged at 4000 rpm for 10 min at +4 oC. The serum samples remaining after centrifugation were separated into Eeppendorf tubes and stored at -80 oC until the tests were performed. During the study, after quality control of the well-dissolved serum samples on the Roche cobas e 601 device (Roche Diagnostics, Germany) used in our routine laboratory, invitro quantitative detection of antibodies (Ig M and G) developed against SARS-CoV-2 spike (S) protein receptor binding domain (RBD) was performed with an immunochemical method (Roche Elecsys Anti-SARS-CoV-2 S Quant) compatible with the cobas e 601 device. The measurement range of the kit is between 0.40-250 U/ml and the manufacturer's recommended result interpretation states that values above 0.80 U/ml should be considered positive (Available at: https://www.fda.gov/media/144037/download). The Sserum neutralizsation of the test was good and acceptable, and sera with results greater than 250 were retested at 1/10 and 1/50 dilutions and evaluated.

Ethical Approval

The study was carried out with the approval of Afyonkarahisar Health Sciences University Clinical Research Ethics Committee (Date: 05.02.2021 and Decision No: 2021/99) and the participants were informed and included with their consent

Statistical Analysis

In the study, categorical variables were presented with numbers and percentages. Confidence intervals of percentages were calculated by Wilson's method (continuity correction). The Aage of the subjects was presented as mean and standard deviation. People with antibody levels >0.8 U/ml were considered

seropositive. Antibody levels of seropositive individuals are were presented as mean, standard deviation, median and IQR. Seropositivity levels between months were determined by Cochran's Q test in individuals with complete antibody measurements at months 2 and 4, and then differences between months were determined by post hoc Dunn's test and the Bbonferroni correction. In addition, the seropositivity levels of individuals with complete antibody measurements at both 2 and 4 months are shown in bar graphs. The statistical significance limit for P value was accepted as 0.05. Statistics were performed with SPSS v.28.0.

Results

A total of 81 people were included in the study;. 51.9% (n=42) of the participants were female and the mean age was 67.2±4.6 years;. 48.1% (n=39) had at least one comorbidity. Of the 81 participants included in the study, 79 could be reached inat the first month, 49 in at the second month, and 12 in at the fourth month after vaccination and antibody measurements could be performed. The characteristics of the participants and antibody levels according to months are presented in Table 1 with 95% confidence intervals.

In the pre-vaccination evaluation, 34.6% of the participants were seropositive. In 23 of the 79 participants evaluated in at the first month, the antibody level was below 0.8U/ml and was considered seronegative. 39.1% (n=9) of these participants were male and 60.9% (n=14) were female. There was no statistically significant difference in terms of gender in those

Table 1. Antibody seropositivity according to the characteristics of the participants and measurement times.

Characteristics	n, %	%95 G.A.
Gender (n=81)		
Woman	42, 51.9	40.5-63.0
Male	39, 48.1	37.0-59.5
Age (n=81, mean+standard deviation)	67.2±4.6	66.2-68.2
Presence of comorbidity (n=81)		
At least one comorbidity	39, 48.1	37.0-59.5
Hypertension	32, 39.5	29.0-51.0
Diabetes	15, 18.5	11.1-29.0
Heart failure	13, 16.5	9.2-26.3
Renal failure	2, 2.5	0.4-9.5
Lung disease	2, 2.5	0.4-9.5
Rheumatic disease	1, 1.2	0.1-7.6
Cerebrovascular accident	1, 1.2	0.1-7.6
Anti Covid-19 antibody seropositivity (n,%)		
Before vaccination (n=81)	28, 34.6	24.6-46.0
1st month (n=79)	56, 70.9	59.4-80.3
2nd month (n=49)	49, 100.0	90.9-100.0
4th month (n=12)	12, 100.0	69.9-100.0
Antibody levels in seropositive individuals (U/ml)	**Mean+standard deviation**	**Interquartile Range (IQR)**
Before vaccination (n=28)	839.9±1714.2	76.7(520.4)
1st month (n=56)	912.0±2284.5	46.6(756.3)
2nd month (n=49)	392.1±949.7	54.4(202.0)
4th month (n=12)	279.2±498.2	48.3(367.2)

Confidence interval calculations were made according to the Wilson method (with continuity correction).

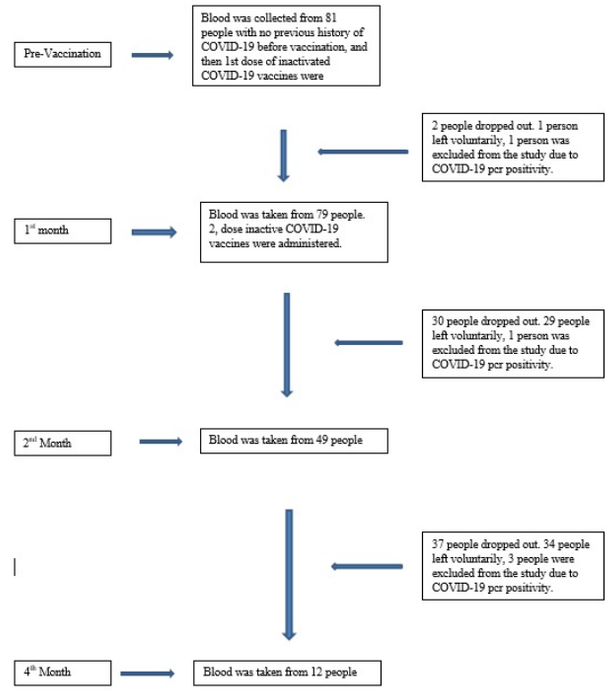

Figure 1. Flowchart.

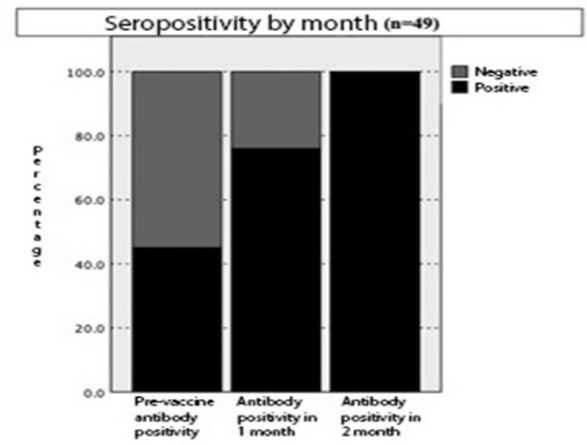

Figure 2. Antibody seropositivity according to the months of the people (n=49) whose antibody measurements were completed as of the 2nd month.

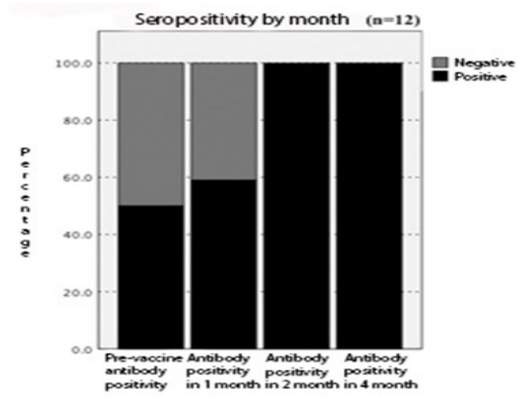

Figure 3. Antibody seropositivity according to months in people (n=12) whose antibody measurements were completed as of the 4th month.

Table 2. Statistical analysis of the differences between antibody seropositivity levels at 1, 2 and 4 months before and after vaccination.

Antibody seropositivity (n, %)	Test Statistic *	Std. Error	Std. Test Statistic	P	Adj.P.**
2nd month (49, 100.0) – 1st month (37, 75.5)	0.245	0.087	2.828	0.005	0.014
2nd month (49, 100.0) – Before vaccination (22, 44.9)	0.551	0.087	6.364	<0.001	0.000
1st month (37, 75.5) – Before vaccination (22, 44.9)	0.306	0.087	3.536	<0.001	0.001
4th month (12, 100.0) - Before vaccination (6, 50.0)	0.500	0.163	3.065	0.002	0.013
4th month (12, 100.0) - 1st month (7, 58.3)	0.417	0.163	2.554	0.011	0.064
2nd month (12, 100.0) - 4th month (12, 100.0)	0.000	0.163	0.000	1.000	1.000

*Post-hoc Dunn test, **Bonferroni correction

who failed to produce sufficient antibodies in the first month (p=0.306). Of the 23 people who were seronegative in the first month, 9 (39.1%) had any comorbidity. There was no statistically significant difference in terms of having comorbidity in seronegative people compared to seropositives (p=379).

As of the 2nd month, antibody measurements (pre-vaccination, 1st month, 2nd month) of a total of 49 individuals were completed and the findings are presented in Figure 2. Seropositivity increased statistically significantly within 2 months compared to the pre-vaccination period (n=49, Cochran Q test=40.7, df=2, p<0.001). By the 4th month, antibody measurements (pre-vaccination, 1st month, 2nd month and 4th month) of a total of 12 individuals were completed and the findings are presented in Figure 3. Seropositivity increased statistically significantly atin 4 months compared to the pre-vaccination period (n=12, Cochran Q test=16.0, df=3, p=0.001).

The differences in seropositivity between pre-vaccination, 1st month, 2nd month and 4th month are presented in Table 2. Antibody seropositivity at in the1st month, 2nd month and 4th month showed a statistically significant increase compared to the pre-vaccination period. In addition, antibody seropositivity inat the 2nd month showed a statistically significant increase compared to the seropositivity inat the 1st month. Between the 2nd and 4th months, there was no change, and the antibody seropositivity at the 2nd and 4th months was determined as 100.0% in individuals who had all antibody measurements completed in both 2 (n=49) and 4 (n=12) months.

Discussion

Serological antibody detection is an indispensable tool in the management of infectious diseases, including diagnosis, determination of protective antibody titre after vaccination and epidemiological assessment of humoral immunity. Low antibody seroprevalence against SARS-CoV-2 indicates that populations are vulnerable to a second wave of infection [16]. This study is very important because it is one of the few studies on CoronaVac administration in elderly individuals in which antibody levels were measured before vaccination, thus providing the opportunity to compare the seropositivity status before vaccination.

Although the elderly who declared that they did not have COVID-19 before vaccination and did not have PCR positivity before vaccination were included in the study, it was determined that 28 (34.6%) people were seropositive when the pre-vaccine antibody levels were examined. This may be explained by the fact that seropositive people had asymptomatic infection without the need to consult a physician before vaccination or cross-reactions that may be seen in the laboratory method. This high seropositivity rate in the patient group without evidence of infection shows the severity and prevalence of the pandemic in this period and the importance of protecting this group, which has a high risk of developing severe disease, by immunizsation [16].

In this study, antibody levels of people over the age of 60 were monitored before and for four months after the CoronaVac vaccine administration. It was found that both seropositivity rates and mean antibody levels of seropositive individuals increased in the first and second months, but in the fourth month, although there was no increase in the mean antibody levels of seropositives individuals, the mean antibody level in seropositive individuals decreased compared to the second month. Decreasing antibody levels over time suggest that reminder doses are necessary to protect this age group against the risk of severe Sars-CoV-2 infection.

It is known that neutralizsing antibodies formed after vaccination are protective against SARS-CoV-2 infection and that these antibodies prevent the virus from binding to the ACE-2 receptor of human cells by binding to the S protein [17]. CoronaVac is an inactivated SARS-CoV-2 vaccine shown to be 65.9%, 87.5%, 90.3%, and 86.3% effective in preventing COVID-19 symptoms, hospitalizsations, intensive care admissions, and COVID-19-related deaths, respectively. However, the efficacy of vaccines may be impaired by the presence of previously recognizsed diseases or pathologies. In addition, the severity of the disease may be even more pronounced in the elderly, as their immune systems are more dysfunctional than in younger people [18]. Considering the presence of increased comorbidity with age, elderly individuals show more severe symptoms of SARS-CoV-2 infection due to the development of a defective immune response. Especially hypertension, obesity, diabetes, cardiovascular diseases and malignancy cause significantly increased mortality and morbidity rates in this age group [19]. In this study, 48.1% of the patients had at least one comorbidity and hypertension, diabetes and heart failure were found most frequently, respectively. These high comorbidity rates indicate the importance of protecting people over the age of 60 against Sars-CoV-2 infection. Unfortunately, it has been observed that the mask, distance, hand hygiene and other restrictive measures implemented at the beginning of the outbreak were not effective enough in the prevention of the spread and control of the infection. This situation once again demonstrates the necessity of appropriate immunizsation with effective vaccines. In Brazil, where CoronaVac is the most widely used vaccine, the immunogenicity of the vaccine in elderly individuals is still little known, despite the finding that the COVID-19 mortality rate decreased after vaccination in people over 70 years of age and provided protection for this age group [20]. B cells, which are responsible for responses such as the production of antibodies

and secretion of cytokines, are the most important mediators of acquired immunity. Although differences in the function of B cells and plasma cells have not yet been sufficiently analyzsed in terms of COVID-19 or coronavirus infections, changes occur that are valid for any pathogen occur [21]. Although some studies have reported up to 98% seroconversion in vaccinated people, anti-spike antibody titres have been observed to be significantly lower among people aged 60 years and older [20,21]. It has been reported that aging is associated with a decline in immune function and this process, known as immune aging, generally leads to deterioration of the response to vaccines in older adults. As a matter of fact, when immune responses to inactivated vaccines such as influenza were analyzsed, it was found that elderly individuals had significantly lower protection, and vaccine efficacy in these individuals ranged between 17% and 51%, whereas this rate increased up to 90% in young individuals [20]. Our observation that post-vaccination antibody levels decrease over time in this age group is consistent with studies showing that the elderly exhibit poorer responses to the trivalent inactivated influenza vaccine [22].

In a nationwide evaluation of vaccine efficacy involving 25,639,346 Brazilians vaccinated with CoronaVac, it was found that protection against hospitalizsation, intensive care unit admission and death decreased in persons older than 79 years, and there was an increase in the rate of hospitalizsation in persons older than 80 years sixty days after the second dose of vaccination. Compared with individuals under 60 years of age vaccinated on the same calendar date, individuals over 90 years of age had higher rates of COVID-19-related hospitalizsations. When the entire post-vaccination period was considered, hospitalisation rates remained low among individuals under 60 years of age despite higher SARS-CoV-2 exposure, in contrast to the gradually increasing rates among individuals over 90 years of age [23]. These results support our belief that reminder doses are needed in older individuals.

Limitations

The major limitation of this study is the large reduction in the number of participants at month 4 months. Therefore, our analyses were interpreted by comparing the mean antibody levels of seropositive individuals. In order to make a clearer interpretation on this subject, studies with more participants and in which the number of participants did not decrease in repeated measurements are needed.

Conclusion

Our study showed that an adequate antibody response was obtained with the CoronaVac vaccine in individuals over 60 years of age, but the antibody levels obtained decreased over time. According to these results, vaccination against SARS-CoV-2 in individuals over 60 years of age with inactivated virus vaccines produces an effective antibody response, but additional doses are required for the sustainability of the antibody response.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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